Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes-*RHOA*, *RCHY1* and *RSU1*

¹Liu Yonggang and ²Xia Xueshan

¹College of Animal Science and Technology,

Yunnan Agricultural University, 650201 Kunming, China

²Faculty of Life Science and Technology,

Kunming University of Science and Technology, 650224 Kunming, China

Abstract: The complete coding sequences of three sheep genes-*RHOA*, *RCHY1* and *RSU1* were amplified using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The sheep *RHOA* gene encodes a protein of 193 amino acids that shares high homology with the ras homolog gene family, member A (RHOA) proteins of fifteen species- cattle (100%), rat (99%), human (100%), mouse (99%), chicken (99%), dog (99%), turkey (99%), sumatran orangutan (99%), poephila guttata (99%), Atlantic salmon (97%), green anole (96%), rainbow trout (96%), African clawed frog (96%), zebrafish (96%) and Western clawed frog (95%). The sheep *RCHY1* gene encodes a protein of 261 amino acids that shares high homology with the ring finger and CHY zinc finger domain containing 1 (RCHY1) proteins of nine species-cattle (94%), giant panda (92%), pig (91%), human (90%), white-tufted-ear marmoset (90%), chimpanzee (90%), rabbit (89%), mouse (86%) and rat (84%). The sheep *RSU1* gene encodes a protein of 277 amino acids that shares high homology with the ras suppressor protein 1 (RSU1) proteins of twelve species-cattle (99%), chimpanzee (98%), dog (99%), human (98%), giant panda (98%), horse (98%), rabbit (98%), chicken (96%), mouse (97%), African clawed frog (94%), Western-clawed-frog (94%) and zebrafish (91%). Finally, these three novel sheep genes were assigned to GeneIDs: 100302078, 100302546 and 100302548. The phylogenetic analysis revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes all have a closer genetic relationship with the *RHOA*, *RCHY1* and *RSU1* genes of cattle. Tissue expression profile analysis demonstrated that sheep *RHOA*, *RCHY1* and *RSU1* genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, *RHOA*, *RCHY1*, *RSU1*, tissue expression, China

INTRODUCTION

Ras homolog gene family, member A (RHOA) belongs to the RhoA-like sub-family. The RhoA-like sub-family consists of RHOA, RHOB and RHOC. Latest researches demonstrated that RHOA promotes the formation of stress fibers and focal adhesions, regulating cell shape, attachment and motility (Su *et al.*, 2006). RHOA is vital for muscle contraction in vascular smooth muscle cells, RHOA plays a key role in cell contraction, differentiation, migration and proliferation (Peyton *et al.*, 2008). RHOA activities appear to be elaborately regulated in a time- and space-dependent manner to control cytoskeletal changes (Maekawa *et al.*, 1999; Ohta *et al.*, 1999; Saeki *et al.*, 2005). Ring finger and CHY zinc finger domain containing 1 (RCHY1), a member of proteins containing the ring finger and CHY zinc finger domain has ubiquitin ligase activity. It mediates E3-dependent

ubiquitination and proteasomal degradation of target proteins including TP53, HDAC1 and CDKN1B thus, regulating their levels and cell cycle progression (Wu *et al.*, 2010; Yan *et al.*, 2010; Shloush *et al.*, 2011).

Ras Suppressor protein 1 (*RSU1*) gene encodes a protein that is involved in the ras signal transduction pathway, growth inhibition and nerve-growth factor induced differentiation processes as determined in mouse and human cell line studies. In mouse, the encoded protein was initially isolated based on its ability to inhibit v-Ras transformation. Multiple alternatively spliced transcript variants for this gene have been reported; one of these variants was found only in glioma tumors (Chunduru *et al.*, 2002; Dougherty *et al.*, 2005, 2008).

As mentioned before, *RHOA*, *RCHY1* and *RSU1* genes are three genes which have important functions. Until today, *RHOA*, *RCHY1* and *RSU1* genes had been reported in human and other animals but the sheep *RHOA*,

RCHY1 and *RSU1* genes have not been reported yet. In present experiment, the researchers will isolate the coding sequences of sheep *RHOA*, *RCHY1* and *RSU1* genes based on the coding sequence information of *RHOA*, *RCHY1* and *RSU1* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform sequence and tissue expression profile analysis for these genes. These will establish the primary foundation of understanding these three sheep genes.

MATERIALS AND METHODS

Animals and sample preparation: Five adult Yunnan local sheep were slaughtered. Spleen, skin, lung, fat, muscle, heart, liver, kidney and ovary samples were collected, frozen in liquid nitrogen and then stored at -80°C . The total RNA was extracted using the total RNA extraction kit (Gibco, USA). First-strand cDNA synthesis was performed as that described by Liu *et al.* (2004). These first-strand cDNA samples were used to perform RT-PCR for the isolation of sheep *RHOA*, *RCHY1* and *RSU1* genes and for the tissue expression profile analysis.

Isolation of the sheep *RHOA*, *RCHY1* and *RSU1* genes:

The primers for sheep *RHOA* gene isolation were designed based on the coding sequence information of human *RHOA* gene and its highly homologous sheep EST sequences: DY503081 and EE758341. Similarly, the primers for sheep *RCHY1* gene isolation were designed based on the coding sequence information from human *RCHY1* gene and its highly homologous sheep EST sequences; DY519128 and EE754481. The primers for sheep *RSU1* gene isolation were designed based on the coding sequence information from human and mouse *RSU1* genes and their highly homologous sheep EST sequences; DY495018 and FE027712. These primer sequences and their annealing temperature for RT-PCR reaction were shown in Table 1. The RT-PCR was performed to isolate these three sheep genes using the pooled cDNAs from different tissues above. The 25 μL reaction system was; 2.0 μL cDNA, 2.5 μL 2 mM mixed dNTPs, 2.5 μL 10 $\times$ Taq DNA polymerase buffer, 2.5 μL 25 mM MgCl_2 , 2.0 μL 10 μM forward primer, 2.0 μL 10 μM reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 μL) and 9.5 μL sterile water. The PCR program initially started with a 94°C denaturation for 4 min followed by 35 cycles of $94^{\circ}\text{C}/50$ sec, $\text{Ta}^{\circ}\text{C}/50$ sec, $72^{\circ}\text{C}/50$ sec then 72°C extension for 10 min, finally 4°C to terminate the reaction (Table 1).

These PCR products for sheep *RHOA*, *RCHY1* and *RSU1* genes were then cloned into PMD18-T vector and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones were sequenced for every gene.

Table 1: Primers for sheep *RHOA*, *RCHY1*, *RSU1* and *beta-actin* genes and their annealing Temperature ($\text{Ta}^{\circ}\text{C}$)

Genes	Primer sequence	$\text{Ta}^{\circ}\text{C}$
<i>RHOA</i>	Forward 5'- ATGGCTGCCATCCGGAAG-3'	61
	Reverse: 5'-TCACAAGACAAGGCACCCAG-3'	
<i>RCHY1</i>	Forward 5'-ATGGCTTCCTCGACGCTG-3'	61
	Reverse: 5'-TCATTGCTGATCTAATGAAATT-3'	
<i>RSU1</i>	Forward: 5'-ATGTCCAAGTCTCTGAAA-3'	52
	Reverse: 5'-TTATTATTCTTGGCTGCC-3'	
<i>Beta-actin</i>	Forward: 5'-CTTGATGTCACGGACGATTT-3'	56
	Reverse: 5'- CACGGCATTGTCAACCACT-3'	

RT-PCR for tissue expression profile analysis: RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Gao, 2009; Yonggang and Shizheng, 2009; Liu, 2009). The researchers selected the housekeeping gene *beta-actin* (Accession No.: NM_001009784) as a positive control. The primers of sheep *RHOA*, *RCHY1* and *RSU1* genes which were used to perform the RT-PCR for tissue expression profile analysis were same as the primers for isolation RT-PCR above. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 μL reaction system was: 1 μL cDNA ($100 \text{ ng } \mu\text{L}^{-1}$), 5 pmoles each oligonucleotide primer, 2.5 μL , 2 mmol L^{-1} mixed dNTPs, 2.5 μL 10 $\times$ Taq DNA polymerase buffer, 2.5 μL 25 mmol L^{-1} MgCl_2 , 1.0 unit of Taq DNA polymerase and finally add sterile water to volume 25 μL . The PCR program initially started with a 94°C denaturation for 4 min followed by 25 cycles of $94^{\circ}\text{C}/50$ sec, $\text{Ta}^{\circ}\text{C}/50$ sec, $72^{\circ}\text{C}/50$ sec then 72°C extension for 10 min, finally 4°C to terminate the reaction (Table 1).

Sequence analysis: The cDNA sequence prediction was conducted using GenScan software (<http://genes.mit.edu/GENSCAN.html>). The protein prediction and analysis were performed using BLAST tool at the National Center for Biotechnology Information (NCBI) server (<http://www.ncbi.nlm.nih.gov/BLAST>) and the ClustalW software (<http://www.ebi.ac.uk/clustalw>).

RESULTS AND DISCUSSION

RT-PCR results for sheep *RHOA*, *RCHY1* and *RSU1* genes: Through RT-PCR with pooled tissue cDNAs, for sheep *RHOA*, *RCHY1* and *RSU1* genes, the resulting PCR products were 582, 786 and 834 bp (Fig. 1).

Sequence analysis: These cDNA nucleotide sequence analysis using the BLAST software at NCBI server (<http://www.ncbi.nlm.nih.gov/BLAST>) revealed that these three genes were not homologous to any of the known sheep genes and they were then deposited into the GenBank database (Accession No.: FJ943984, FJ937958 and FJ937963). The sequence prediction was carried out

Fig. 1: RT-PCR results for sheep *RHOA*, *RCHY1* and *RSU1* genes. M: DL 2000 DNA markers; 1: PCR product for sheep *RHOA* gene; 2: PCR product for sheep *RCHY1* gene; 3: PCR product for sheep *RSU1* gene

using the GenScan software and results showed that the 582, 786 and 834 bp cDNA sequences represent three single genes which encoded 193, 261 and 277 amino acids, respectively. Finally, these three novel sheep genes were assigned to GeneIDs; 100302078, 100302546 and 100302548.

Further BLAST analysis of these proteins revealed that the sheep *RHOA* protein has high homology with the ras homolog gene family, member A (*RHOA*) proteins of fifteen species-cattle (Accession No.: NP_788818; 100%), rat (Accession No.: NP_476473; 99%), human (Accession No.: NP_001655; 100%), mouse (Accession No.: NP_058082; 99%), chicken (Accession No.: NP_990035; 99%), dog (Accession No.: NP_001003273; 99%), Turkey (Accession No.: ADX97247; 99%) sumatran orangutan (Accession No.: NP_001124755; 99%), poephila guttata (Accession No.: ACH45076; 99%), Atlantic salmon (Accession No.: ACI33725; 97%), green anole (Accession No.: XP_003216698; 96%), rainbow trout (Accession No.: NP_001158532; 96%), African clawed frog (Accession No.: AD40671; 96%), zebrafish (Accession No.: NP_998515; 96%) and Western clawed frog (Accession No.: CAJ81715; 95%) (Fig. 2).

The sheep *RCHY1* protein has high homology with the ring finger and CHY zinc finger domain containing 1 (*RCHY1*) proteins of nine species-cattle (Accession No.: NP_001077223; 94%), giant panda (Accession No.: XP_002919200; 92%), pig (Accession No.: NP_001090959; 91%), human (Accession No.: NP_056251; 90%), white-tufted-ear marmoset (Accession No.: XP_002745761; 90%), chimpanzee (Accession No.: XP_517222; 90%), rabbit (Accession No.: XP_002717057; 89%), mouse (Accession No.: NP_0808337; 86%) and rat (Accession No.: NP_001007619; 84%) (Fig. 3).

The sheep *RSU1* protein has high homology with the ras suppressor protein 1 (*RSU1*) proteins of twelve species-cattle (Accession No.: NP_001035691; 99%), chimpanzee (Accession No.: XP_001151460; 98%), dog (Accession No.: XP_535177; 99%), human (Accession No.: NP_036557; 98%), giant panda (Accession No.: XP_002918673; 98%), horse (Accession No.: XP_001498103; 98%), rabbit (Accession No.: XP_002717469; 98%), chicken (Accession No.: NP_001186520; 96%), mouse (Accession No.: NP_033131; 97%), African clawed frog (Accession No.: NP_001085943; 94%), Western-clawed-frog (Accession No.: NP_001015695; 94%) and zebrafish (Accession No.: XP_002666722; 91%) (Fig. 4).

Based on the results of the alignment of *RHOA*, *RCHY1* and *RSU1* proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software as shown in Fig. 5-7. The phylogenetic analysis revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes all have a closer genetic relationship with the *RHOA*, *RCHY1* and *RSU1* genes of cattle.

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes are all generally but differentially expressed in tissues including spleen, lung, muscle, kidney, ovary, skin, liver, heart and fat (Fig. 8).

In the current study, the researchers firstly get the coding sequences of sheep *RHOA*, *RCHY1* and *RSU1* genes by RT-PCR. With the development of modern bioinformatics, establishment of specific sheep NCBI EST database and different convenient analysis tools, researchers can easily find the useful ESTs which were highly homologous to the coding sequences of human

Rat	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Mouse	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Sheep	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Cattle	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Human	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Sumatran orangutan	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Dog	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Poephila guttata	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Chicken	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Turkey	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Zebrafish	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
Green anole	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Rainbow trout	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
Atlantic salmon	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
African clawed frog	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Western clawed frog	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
	*****:*****
Rat	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Mouse	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Sheep	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Cattle	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Human	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Sumatran orangutan	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Dog	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Poephila guttata	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLQNIPEKWTPEVKHFCPNVPIILVGNKKD
Chicken	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Turkey	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Zebrafish	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Green anole	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Rainbow trout	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Atlantic salmon	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
African clawed frog	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Western clawed frog	AGQEDYDRLRPLSYPDTDVILMCFSDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
	*****:*****
Rat	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Mouse	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Sheep	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Cattle	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Human	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Sumatran orangutan	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Dog	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Poephila guttata	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Chicken	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Turkey	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Zebrafish	LRNDEHTRRELAKMKQEPVKPEEGRDMANRINAFGYLECSAKTKDGVREVFEMATRAALQ
Green anole	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYLECSAKTKDGVREVFEMATRAALQ
Rainbow trout	LRNDEHTRRELAKMKQEPVKPEEGRDMANRISAFGYMECSAKTKDGVREVFEMATRAALQ
Atlantic salmon	LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKSKDGVREVFEMATRAALQ
African clawed frog	LRNDEHTRRELTKMKQEPVKPEEGRDMANRISAYAYMECSAKTKDGVREVFELATRAALQ
Western clawed frog	LRNDEHTRRELTKMKQEPVKPEEGRDMANRISAYAYMECSAKTKDGVREVFELATRAALQ
	*****:*****
Rat	ARRGKKKSGCLIL
Mouse	ARRGKKKSGCLIL
Sheep	ARRGKKKSGCLVL
Cattle	ARRGKKKSGCLVL
Human	ARRGKKKSGCLVL
Sumatran orangutan	ARRGKKKSGRLVL
Dog	ARRGKKKSGCLVL
Poephila guttata	ARRGKKKSGCLFL
Chicken	ARRGKKKSGCLLL
Turkey	ARRGKKKSGCLLL
Zebrafish	ARRGKKKSGCLLL
Green anole	AKRGRKKTSCQLL
Rainbow trout	ARRGKPRNKCLLL
Atlantic salmon	ARRGKKSNKCLLL
African clawed frog	ARRGKKKPRCLLI
Western clawed frog	ARRGKKKTTCLLI
	*:: .:

Fig. 2: The alignment of the protein encoded by sheep *RHOA* gene and fifteen other kinds *RHOA* proteins

257

Fig. 5: The phylogenetic analysis for sixteen kinds of *RHOA* genes

Fig. 6: The phylogenetic analysis for ten kinds of *RCHY1* genes

Fig. 7: The phylogenetic analysis for thirteen kinds of *RSU1* genes

genes. Based on these sheep EST sequences, the researchers can obtain the complete coding sequences of some novel sheep genes through the some experimental methods such as RT-PCR. From the clone and sequence analysis of sheep *RHOA*, *RCHY1* and *RSU1* genes, it could be seen that this is an effective method to isolate some novel sheep genes.

Through sequence analysis, the researchers found that the encoding protein of the sheep *RHOA*, *RCHY1* and *RSU1* genes are highly homologous with *RHOA*, *RCHY1* and *RSU1* proteins of human and some other animals. This implied that the *RHOA*, *RCHY1* and *RSU1* genes were highly conserved in some species and the sheep *RHOA*, *RCHY1* and *RSU1* genes will have similar

Fig. 8: Tissue expression distribution of sheep *RHOA*, *RCHY1* and *RSU1* genes. The beta-actin expression is the internal control. 1: muscle; 2: heart; 3: lung; 4: spleen; 5: skin; 6: fat; 7: liver; 8: kidney; 9: ovary

functions as the *RHOA*, *RCHY1* and *RSU1* genes of human and other animals. The researchers also found that the sheep *RHOA*, *RCHY1* and *RSU1* proteins do not show complete identity to some animals. This implied that the sheep *RHOA*, *RCHY1* and *RSU1* genes will have some differences in functions to those of other animals.

The phylogenetic analysis revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes all have a closer genetic relationship with the *RHOA*, *RCHY1* and *RSU1* genes of cattle. This implied that we can use cattle as a model organism to study the sheep *RHOA*, *RCHY1* and *RSU1* genes or use sheep as a model organism to study the cattle *RHOA*, *RCHY1* and *RSU1* genes.

From the tissue distribution analysis in the experiment, it can be seen that the sheep *RHOA*, *RCHY1* and *RSU1* genes were obviously differentially expressed in some tissues. As the researchers did not study functions at protein levels yet there might be many possible reasons for differential expression of sheep *RHOA*, *RCHY1* and *RSU1* genes. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of sheep *RHOA*, *RCHY1* and *RSU1* genes were presented diversely in different tissues.

CONCLUSION

In this study, the researchers first isolated the sheep *RHOA*, *RCHY1* and *RSU1* genes and performed necessary sequence and tissue expression profile analysis. This established the primary foundation for further insight into these novel sheep genes.

ACKNOWLEDGEMENTS

This research was supported by the Candidates of the Young and Middle Aged Academic and Technical Leaders of Yunnan province.

REFERENCES

- Chunduru, S., H. Kawami, R. Gullick, W.J. Monacci, G. Dougherty and M.L. Cutler, 2002. Identification of an alternatively spliced RNA for the Ras suppressor RSU-1 in human gliomas. *J. Neurooncol.*, 60: 201-211.
- Dougherty, G.W., C. Jose, M. Gimona and M.L. Cutler, 2008. The Rsu-1-PINCH1-ILK complex is regulated by Ras activation in tumor cells. *Eur. J. Cell Biol.*, 87: 721-734.
- Dougherty, G.W., T. Chopp, S.M. Qi and M.L. Cutler, 2005. The Ras suppressor Rsu-1 binds to the LIM 5 domain of the adaptor protein PINCH1 and participates in adhesion-related functions. *Exp. Cell Res.*, 306: 168-179.
- Liu, G.Y. and S.Z. Gao, 2009. Molecular cloning, sequence identification and tissue expression profile of three novel sheep (*Ovis aries*) genes-BCKDHA, NAGA and HEXA. *Biol. Res.*, 42: 69-77.
- Liu, G.Y., 2009. A novel HADHA gene differentially expressed in muscle and other tissues from black-boned vs. ordinary sheep. *Anim. Sci. Pap. Rep.*, 27: 127-137.
- Liu, G.Y., Y.Z. Xiong, C.Y. Deng, B. Zuo and J.H. Zhang, 2004. Comparison of gene expression patterns in *Longissimus dorsi* of pigs between the high-parent heterosis cross combination Landrace×Large White and the mid-parent heterosis cross combination Large White×Meishan. *Asian-Aust. J. Anim. Sci.*, 17: 1192-1196.
- Maekawa, M., T. Ishizaki, S. Boku, N. Watanabe and A. Fujita *et al.*, 1999. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science*, 285: 895-898.
- Ohta, Y., N. Suzuki, S. Nakamura, J.H. Hartwig and T.P. Stossel, 1999. The small GTPase RalA targets filamin to induce filopodia. *Proc. Natl. Acad. Sci. USA.*, 96: 2122-2128.
- Peyton, S.R., P.D. Kim, C.M. Ghajar, D. Seliktar and A.J. Putnam, 2008. The effects of matrix stiffness and RhoA on the phenotypic plasticity of smooth muscle cells in a 3-D biosynthetic hydrogel system. *Biomaterials*, 29: 2597-2607.
- Saeki, N., H. Tokuo and M. Ikebe, 2005. BIG1 is a binding partner of myosin Ixb and regulates its Rho-GTPase activating protein activity. *J. Biol. Chem.*, 280: 10128-10134.
- Shloush, J., J.E. Vlassov, I. Engson, S. Duan and V. Saridakis *et al.*, 2011. Structural and functional comparison of the RING domains of two p53 E3 ligases, Mdm2 and Pirh2. *J. Biol. Chem.*, 286: 4796-4808.
- Su, W., P. Chardin, M. Yamazaki, Y. Kanaho and G. Du, 2006. RhoA-mediated phospholipase D1 signaling is not required for the formation of stress fibers and focal adhesions. *Cell Signal*, 18: 469-478.
- Wu, G., M. Sun, L. Zhang, J. Zhou, Y. Wang and K. Huo, 2010. A novel hPirh2 splicing variant without ubiquitin protein ligase activity interacts with p53 and is down-regulated in hepatocellular carcinoma. *FEBS Lett.*, 584: 2772-2778.
- Yan, J., D. Zhang, Y. Di, H. Shi, H. Rao and K. Huo, 2010. A newly identified Pirh2 substrate SCYL1-BP1 can bind to MDM2 and accelerate MDM2 self-ubiquitination. *FEBS Lett.*, 584: 3275-3278.
- Yonggang, L. and G. Shizheng, 2009. A novel sheep gene, MMP7, differentially expressed in muscles from black-boned sheep and local common sheep. *J. Applied Genet.*, 50: 253-256.